A Laboratory Model for Sjögren's Syndrome

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SJÖGREN'S SYNDROME (S.S.), a disease of unknown etiology, is characterized clinically by xerostomia and keratoconjunctivitis sicca. In more than 50% of the patients, joint lesions typical of rheumatoid arthritis are present. Clinical manifestations of other autoimmune diseases sometimes accompany S.S. Hypergammaglobulinemia is common and rheumatoid factor is present in the serum in most instances.[1] Also frequently found are circulating nonorgan-specific antinuclear and anticytoplasmic antibodies.[2] Halberg *et al.*[3] found circulating antibody against the epithelial cell cytoplasm of the excretory ducts of salivary glands in the serums of 12 of 21 patients with S.S. The immunologic activity of serum globulins is rivaled only by that seen in systemic lupus erythematosus (S.L.E.).[4] The outstanding histologic finding in S.S. is periductal mononuclear cell infiltration of the salivary and lacrimal glands;[5,6] small lymphocytes predominate but large lymphocytes and reticulum cells may also be present.[4] Increased mononuclear cell infiltration and degenerative changes in the secretory cells are present in later stages of the disease.[7]

Attempts to obtain a laboratory model of Sjögren's syndrome have heretofore been unsuccessful.[8] The purpose of this paper is to report a series of abnormalities occurring spontaneously in NZB and NZB $\times$ NZW F_1 mice which resemble the abnormalities found in Sjögren's syndrome.

Materials and Methods

Mice

A colony of NZW and NZB mice procured from Medical Research Council Laboratories, England, in July 1966 was established and the NZB and NZW strains have been individually maintained by brother-sister matings through the fourth generation. NZB $\times$ NZW F_1 hybrids have been obtained from this stock. The NZW mice are relatively normal animals while the NZB mice are notable for the early development of hemolytic anemia.[9] Thymic lesions, glomerulonephritis, and lymphoid hyperplasia have also been described in the NZB strain.[10] The majority of the F_1 hybrids die in about 6–7 months from a severe lupus-like nephritis. Their serum contains antibodies

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reactive with nuclear material, and lupus erythematosus (LE) cells often are demonstrable on blood smears.[11]

Organs and glands from NZB, NZW, and NZB × NZW F_1 mice 2-15 months of age were weighed at necropsy and portions were fixed in Bouin's solution or neutral buffered formalin for histologic examination. Approximately 10 serial sections were cut from 2 levels of each such portion; other pieces were quick-frozen for immunologic studies with fluorescein-labeled antibodies, the results of which will be reported at a later date.

Saliva Tests

Whole saliva was obtained by aspiration from the floor of the mouth immediately following subcutaneous administration of the sialagogue, methacholine hydrochloride, 1.0 mg./kg. Sodium and potassium were measured by atomic absorption spectrophotometry, chloride was determined colorimetrically using a modification of the method of Zall, Fisher, and Garner.[12] Amylase assays followed the Caraway [13] modification of the standard Smith-Roe starch-iodine procedure and total protein was measured by the Folin-Ciocalteu method.[14]

Results

Histopathology

Data were obtained from examination of the major salivary and exorbital lacrimal glands removed from 30 NZW, 18 NZB, and 29 NZB × NZW F_1 mice ranging from 2 to 15 months of age (Tables 1 and 2).

NZW Mice

The glands from 22 of 23 NZW mice 13 months of age or younger were normal (Fig. 1 and 2). One 8-month-old mouse and 3 of 7 mice older than 13 months showed degenerative changes in the submaxillary gland or in both submaxillary and lacrimal glands. These changes closely resembled those described by Andrew[15,16] in the salivary glands of aging rats. Increased connective tissue and fatty tissue deposition with loss of parenchyma, and the appearance of aberrant acinar cells with enlarged atypical nuclei, pyknosis, and cytoplasmic vacuolation were all observed in the lacrimal and submaxillary glands taken from 3 of the 4 aforementioned

Table 1. Occurrence of Lesions in Submaxillary and Lacrimal Glands of NZW Mice

Mice (No.)	Age (mo.)	No. of mice with no lesions	No. of mice with lesions at different sites *		
			SM only	Lac only	SM & Lac
Males					
17	<13	16	0	0	1
2	>13	2	0	0	0
Females					
6	<13	6	0	0	0
5	>13	2	1	0	2

* SM, submaxillary glands; Lac, lacrimal glands.

NZW mice and in the submaxillary glands of the fourth animal. Lumina of the salivary ducts were frequently found to be plugged with an eosinophilic material, and periductal lymphocytic infiltration was noted in the glands of these animals.

NZB and NZB × NZW F_1 Mice

Types of Lesions. The salivary and lacrimal glands of all the NZB and NZB × NZW F_1 hybrids were normal early in life, but at about 4 months of age periductal and periarteriolar infiltration of mononuclear cells consisting of small and large lymphocytes, plasma cells, and primitive cells (herein called reticulum cells) were observed (Fig. 3 and 4). Other investigators have reported 4–5 months as the age of onset for manifestations of autoimmune phenomena in other tissues.[9–11,17–19] The most characteristic early lesion seen in the NZB and NZB × NZW F_1 mice was the multiple small infiltrations of mononucleocytes (Fig. 5). With increasing age in the mice, the areas of mononuclear cell infiltration were seen to increase in number, enlarge, and at times coalesce (Fig. 6). Aggregates of cells resembling lymphoid follicles were occasionally present in the submaxillary glands (Fig. 7). In the parotid glands of many animals of both strains, cells normally contained within lymph nodes were seen to be traversing the capsule and invading the parenchyma of the gland (Fig. 8). Table 2 shows the frequency of occurrence of mononuclear cell

Table 2. Occurrence of Mononuclear Cell Infiltration in Submaxillary and Lacrimal Glands of NZB and NZB × NZW F_1 Mice

Mice (No.)	Age (mo.)	No. of mice with no lesions	No. of mice with lesions at different sites *		
			SM only	Lac only	SM & Lac
NZB					
Males					
2	<4	0	0	2	0
7	>4	0	0	0	7
Females					
1	<4	0	0	1	0
8	>4	0	0	0	8
NZB × NZW F_1					
Males					
2	<4	2	0	0	0
12	>4	0	0	0	12
Females					
1	<4	1	0	0	0
14	>4	0	0	0	14

* SM, submaxillary glands; Lac, lacrimal glands.

infiltration in the submaxillary and lacrimal glands of all the mice examined.

Other pathologic processes seen in the salivary and lacrimal glands of the NZB and NZB × NZW F_1 mice included edematous changes with an increased number of delicate widely separated connective tissue fibers (Fig. 9). Adjacent discrete areas of acinar cells had lost much of their cytoplasmic stainability, giving them a washed-out appearance. However, their nuclei stained normally and there was no deterioration of cell morphology.

Areas of necrosis were identified in the glands of some mice (Fig. 10). Although the general architecture of the glands on cross section was well preserved, circumscribed areas with karyolysis, pyknosis, and nuclear fragmentation were evident. The adjacent regions of the glands were relatively normal and showed little or no cellular infiltration.

Compact epithelial nodules of pale cells of uniform size and with vesicular nuclei (Fig. 11) were seen in some of the lacrimal glands and in some of the submaxillary and parotid glands. The cytoplasm of these cells was light-staining and nongranular. Their nucleoli and peripheral chromatin were much less basophilic than those of the adjacent normal acinar cells. Islands of these cells were observed in serial sections and did not appear to be undergoing any type of degeneration.

The parotid, submaxillary, and lacrimal glands of many of the NZB and NZB × NZW F_1 hybrids in this study contained dense connective tissue which appeared to be replacing acinar cells. These areas of fibrosis contained small isolated groups of secretory cells, ducts, and small blood vessels, all undergoing destruction. The adjacent areas contained few or no infiltrating mononuclear cells and gave no evidence of previous parenchymal cell damage (Fig. 12).

Distribution of Lesions. The frequency and severity of pathologic findings in the lacrimal gland and the major salivary glands of 15 NZB mice and 26 NZB × NZW F_1 mice all older than 4 months varied with respect to age, type of gland, sex, and strain of mouse.

Periductal and perivascular mononucleocyte infiltrations were observed in every lacrimal gland and submaxillary gland taken from these 41 NZB and hybrid mice; the lacrimal glands from almost every animal were more heavily infiltrated than the submaxillary glands from the same animals. Incidence of parotid gland mononuclear cell involvement was considerably less than 100%. Degenerative lesions followed no set pattern: they were present in some glands and absent in others. However, the parotids appeared to be the most commonly and the most severely affected glands. The sublingual glands of the above-mentioned 41 animals showed less than a 33% incidence of mononuclear cell infiltration and no

degenerative changes. Mononucleocyte accumulations, when present in the sublingual glands, were always small and few in number, seldom exceeding 1 or 2 areas per section.

Over 50% of the female NZB and NZB × NZW F_1 mice had between 3 and 9 areas of mononuclear cell infiltration per section of submaxillary or lacrimal gland, whereas less than 15% of the males had more than 3 infiltrative lesions per section. Within each strain of mice, for any given age group, females appeared to develop more extensive lesions than males.

The glandular lesions seen in the NZB × NZW F_1 hybrids were, in general, more extensive than those in the NZB mice, but the outstanding feature in the glands of the hybrids was the increased mitotic activity of the mononucleocytes (Fig. 13). The mononuclear cells in the glands from both strains varied widely in relative numbers of small and large lymphocytes, plasma cells, and reticulum cells, not only from one animal to another, but from one area to another within a given gland.

Saliva. The results of tests on the saliva from 13 NZW mice, 6 NZB × NZW F_1 mice, and 7 NZB mice revealed no significant differences in sodium, potassium, or chloride levels (Table 3). The amylase concentration of saliva from NZB and NZB × NZW F_1 animals showed a mean figure less than half that of the amylase concentration of saliva obtained from the NZW mice. There was no appreciable difference between the amylase concentration of the saliva from the NZB and the NZB × NZW F_1 animals. The mean value for total protein of the combined NZB and NZB × NZW F_1 group was higher than that of NZW mice, but this was due to high values for the saliva from the NZB × NZW F_1 mice; the NZB mice gave values similar to those obtained for the NZW mice (Table 3). Samples of saliva are now being taken for electrophoretic studies to determine the nature of the proteins in the saliva.

Discussion

The cause of the lesions in the lacrimal and salivary glands of man which characterize Sjögren's syndrome is unknown. Equally obscure is the reason for the frequent association of this syndrome with other auto-

Table 3. Mean Values for Stimulated Whole Saliva from NZW, NZB, and NZB × NZW F_1 Mice

Strain	No.	Amylase *	Na †	K †	Cl †	Prot. conc.†
NZW	13	600	205	152	321	264
NZB	7	294	173	165	313	247
&						316
NZB × NZW F_1	6					459

* In U./ml.
† In mg./100 ml.

immune disturbances. The typical lesions in both salivary and lacrimal glands consist of periductal or perivascular mononuclear cell infiltrations usually sharply demarcated from adjacent normal acinar tissue. Reticulum cells and plasma cells are seen together with the accumulation of lymphocytes. Islands of epithelial cells (generally believed to be derived from the multiplication of duct cells) extending into and occluding the lumen of the duct have been reported in some cases.[5] Although the lobular architecture of the gland is preserved for the most part, with foci of infiltration confined to individual lobules, widespread involvement is sometimes seen, in which case the gland or large portions of it can scarcely be recognized. De novo formation of germinal centers similar to those seen in normal lymph nodes has been reported.[20] Areas of atrophy, fibrosis, and deposition of hyalin-like material does occur.[7]

The NZB and NZB × NZW F_1 strains of mice have been found to exhibit pathologic changes in the salivary and lacrimal glands which are remarkably similar to those encountered in the human disease. Histologic examination reveals glands containing lesions which arise as perivascular or periductal mononuclear cell infiltrations. Those lesions in the salivary and lacrimal glands of the hybrid strain typically show a large number of cells in various phases of mitosis. Later stages lead to widespread gland destruction accompanied by continued mononuclear cell accumulation and/or one or more types of degenerative lesions with connective tissue proliferation. Not infrequently, several distinct kinds of parenchymal destruction are seen to be occurring simultaneously, not only in different glands of the same animal but in different areas of the same gland.

A method for obtaining satisfactory amounts of saliva from mice has been described, and a deficiency in amylase has been found in the strains of animals which have lesions of the salivary glands. This is not associated with a drop in total protein. The discrepancy between amylase concentration and the total protein concentration is being investigated.

Summary

A natural model of Sjögren's syndrome occurs in NZB and NZB × NZW F_1 mice which spontaneously develop many of the features seen in the human disease. The striking similarity between the murine and human diseases is borne out by histopathologic findings and associated autoimmune disorders.

A study of the major salivary and the lacrimal glands of 30 NZW, 18 NZB, and 29 NZB × NZW F_1 mice aged 2–15 months revealed the following:

1. The salivary and the lacrimal glands of 30 NZW mice were essentially normal on histologic examination.

2. The salivary and lacrimal glands of 18 NZB and 29 NZB × NZW F_1 mice showed comparable types of pathologic changes arising in the fourth month and progressing in severity with age. The lesions are characterized by mononuclear cell infiltrations. Epithelial cell nodules are frequently seen. The infiltrative lesions arise about the intralobular ducts and small blood vessels. Also seen are edematous changes, necrosis, and connective tissue replacement of parenchyma. Glandular lesions were found to be more severe in females than in males and more severe in the NZB × NZW F_1 mice than in the NZB mice. The disease process was more advanced in the hybrids than in the NZB animals of corresponding ages.

3. The mean values for amylase concentration in saliva from the NZB and the NZB × NZW F_1 mice were half the mean value for the salivary amylase concentration from the NZW mice. The mean value for total protein of the saliva from the NZB × NZW F_1 hybrids was almost twice the values obtained from saliva from the NZB and the NZW strains, which were comparable to each other.

It is concluded that the pathologic changes occurring in the NZB and NZB × NZW F_1 mice are similar in most respects to those which characterize Sjögren's syndrome in man.

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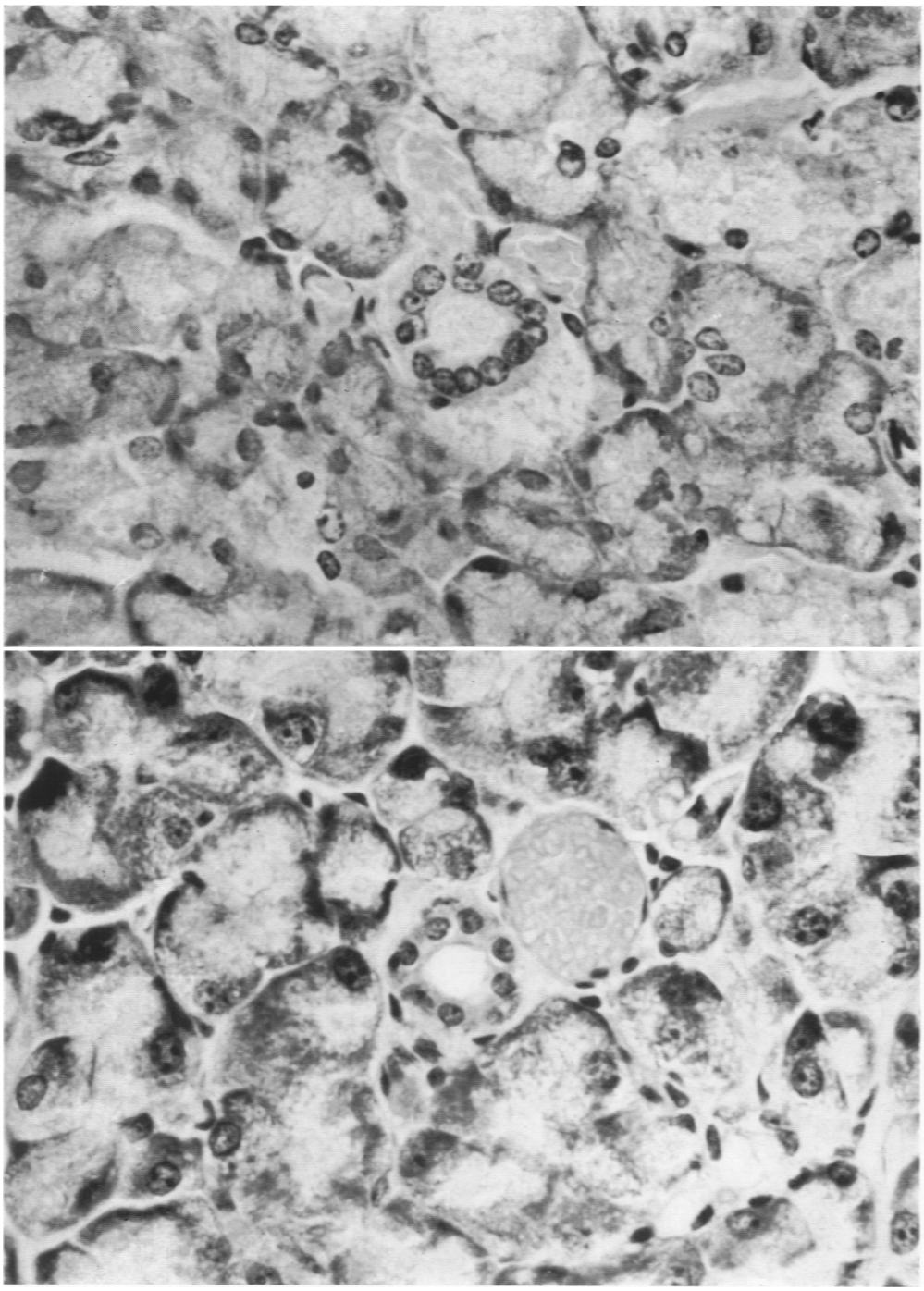
Legends for Figures

Photomicrographs are of sections stained with hematoxylin and eosin.

Fig. 1. Normal submaxillary gland with duct in center of field. NZW female, 6½ months old. × 650.

Fig. 2. Normal lacrimal gland, NZW male, 8 months old. × 650.



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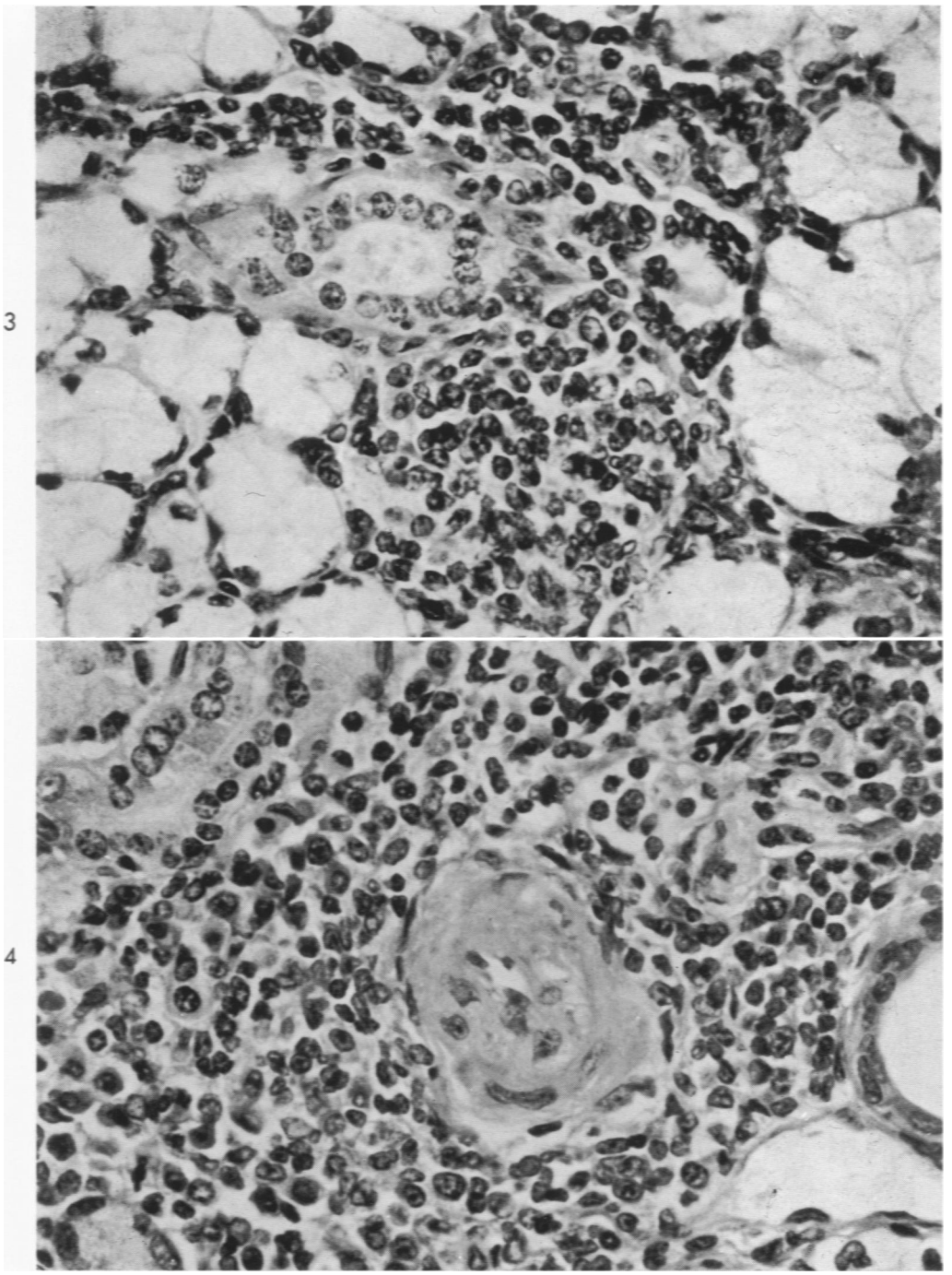

Fig. 3. Sublingual gland. Small area of infiltrating cells. NZB male, 10 months old. × 650. **Fig. 4.** Submaxillary gland. Infiltration around small vessel with thickened walls. NZB × NZW F_1 female, 7½ months old. × 650.

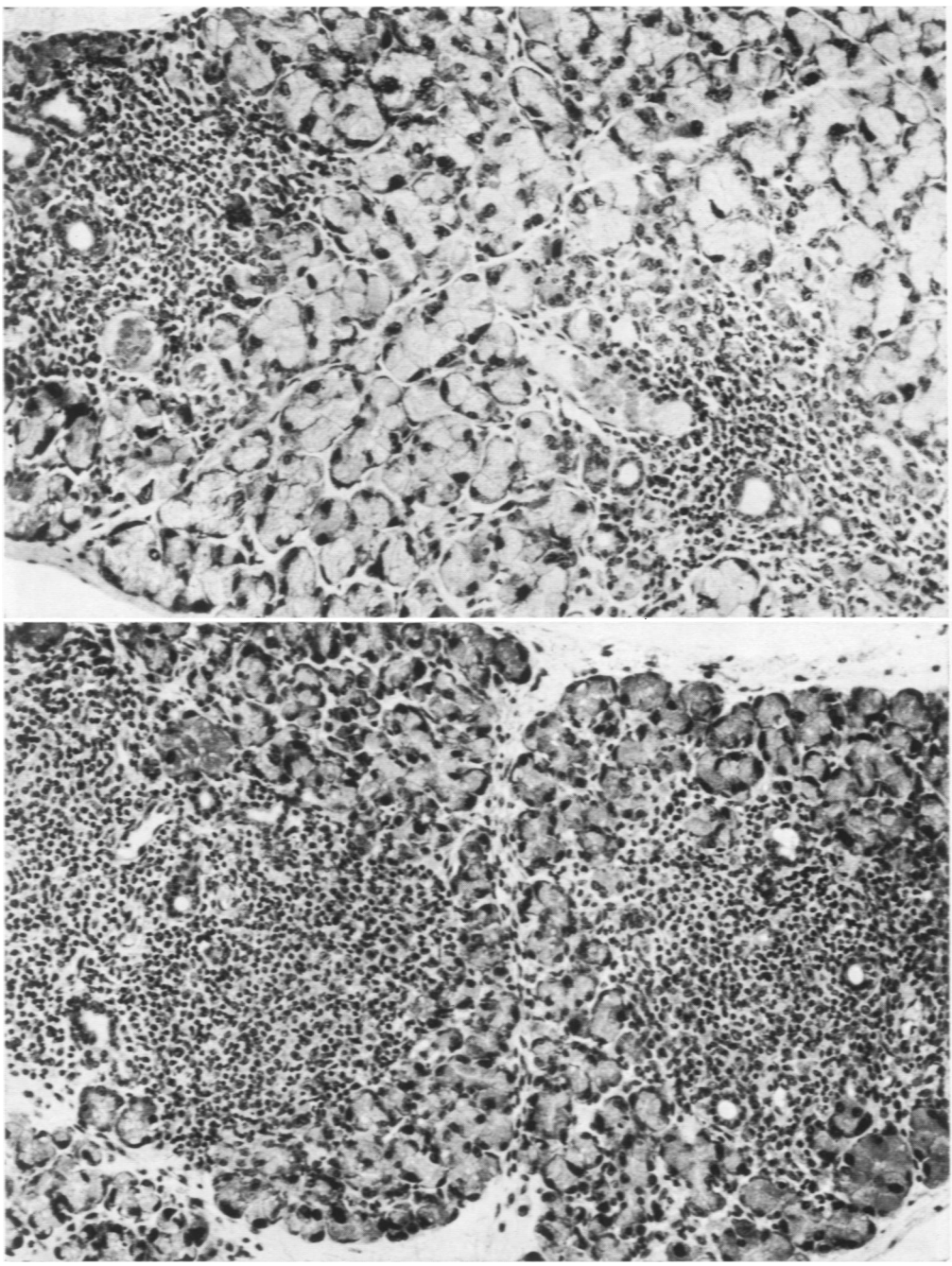

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Fig. 5. Lacrimal gland. Two small periductal areas of mononuclear cell infiltration. Note characteristic paucity of connective tissue. NZB female, 9 months old. × 210. **Fig. 6.** Lacrimal gland. Extension of areas of infiltration (to point of coalescence at left side of field). Compare degree of involvement to age ratio with that for mouse in Fig. 5. NZB × NZW F_1 hybrid female, 7 months old. × 210.

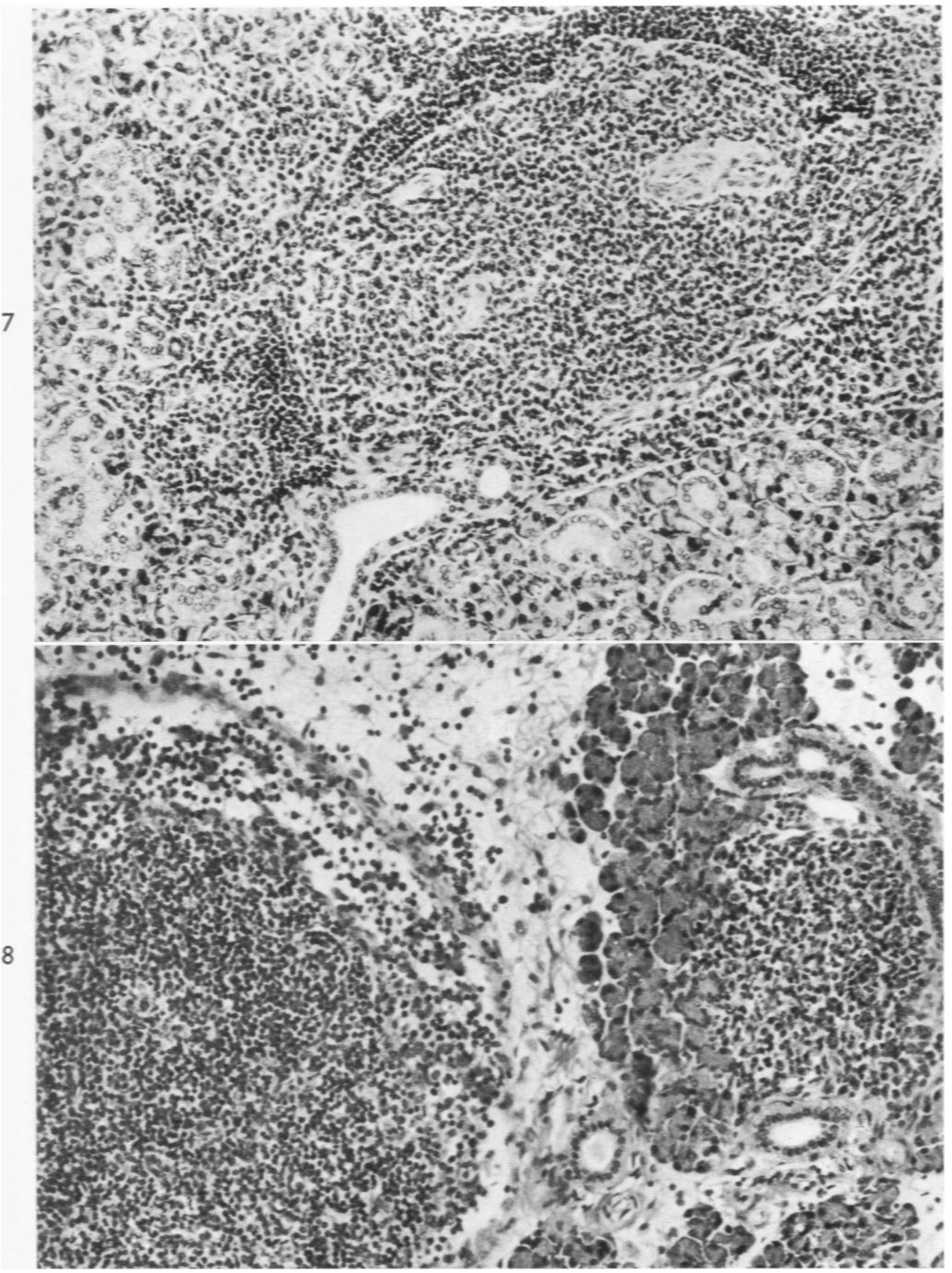

Fig. 7. Submaxillary gland. Infiltration giving appearance of de novo lymphoid follicle formation. NZB × NZW F_1 male, 7 months old. × 210. **Fig. 8.** Parotid gland with infiltration on right, lymph node on left. Note lymphocytes within and outside of capsule of node. NZB × NZW F_1 female, 7½ months old. × 210.

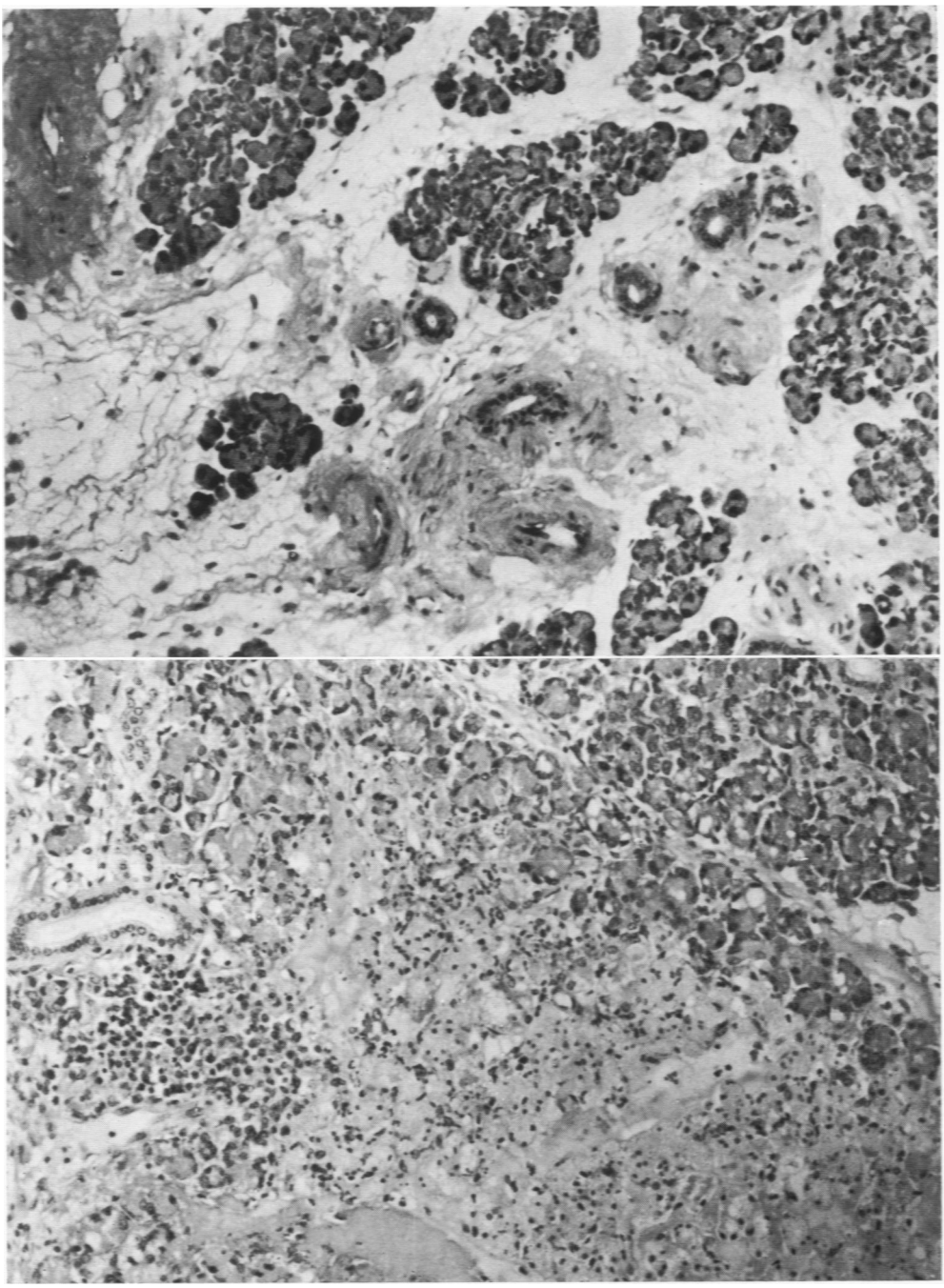

Fig. 9. Parotid gland. Edematous changes and proliferation of connective tissue with destruction of parenchyma. NZB × NZW F_1 female, 7 months old. × 210. **Fig. 10.** Parotid gland. Necrosis is present. Note relatively normal cells in upper right of field. (Animal was sacrificed immediately prior to necropsy.) NZB × NZW F_1 female, 7½ months old. × 210.

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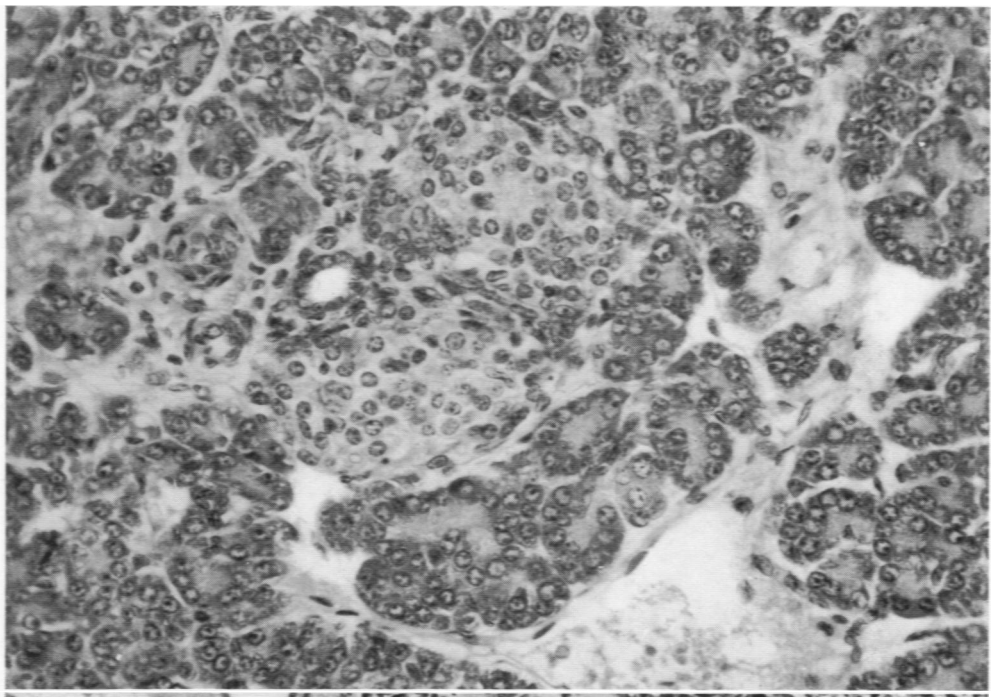

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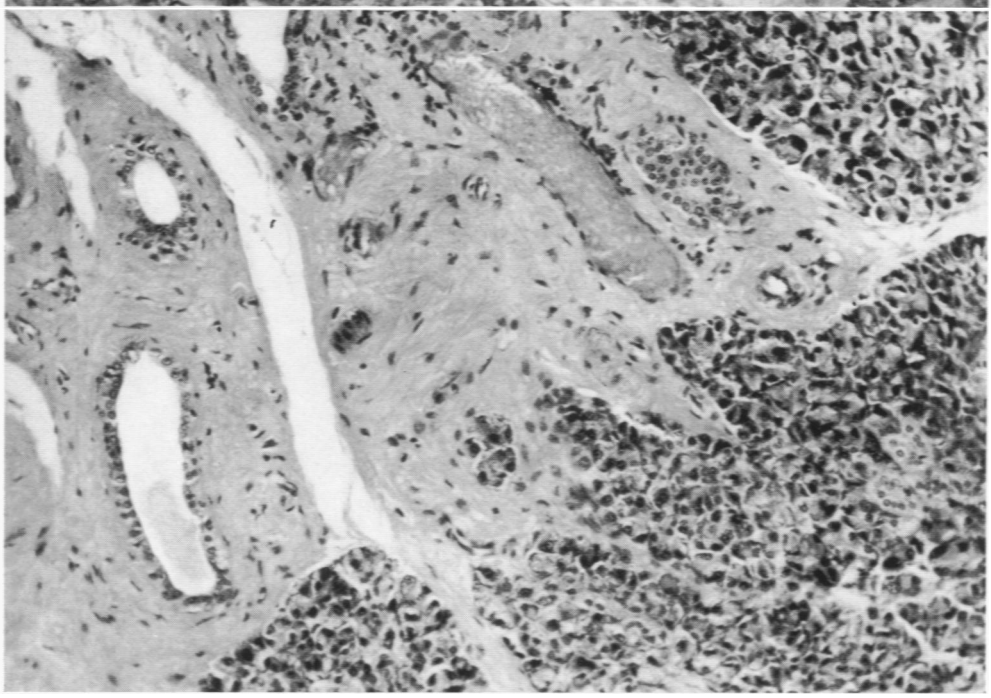

Fig. 11. Lacrimal gland. Small islands of cells with pale vesicular nuclei. NZB × NZW F_1 female, 6½ months old. × 420. **Fig. 12.** Parotid gland. Dense connective tissue replacing acinar tissue. NZB × NZW F_1 female, 7½ months old. × 210.

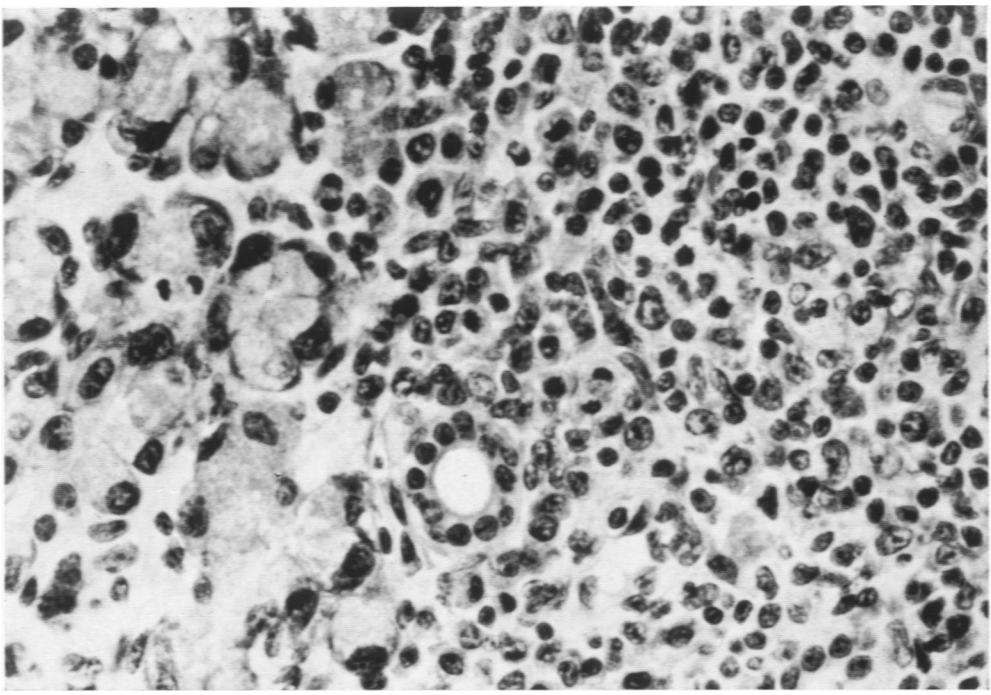

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Fig. 13. Lacrimal gland. Several cells undergoing mitotic division. NZB × NZW F_1 female, 7 months old. × 650.